

# FURTHER OBSERVATIONS ON SPERMATOZOAL MORPHOLOGY AND MALE REPRODUCTIVE TRACT ANATOMY OF *PSEUDOMYS* AND *NOTOMYS* SPECIES (MAMMALIA: RODENTIA)

by W. G. BREED\*

## Summary

BREED, W. G. (1980) Further observations on spermatozoal morphology and male reproductive tract anatomy of *Pseudomys* and *Notomys* species (Mammalia: Rodentia). *Trans. R. Soc. S. Aust.* **104**(3), 51-55, 30 May, 1980.

Spermatozoa of *Notomys cervinus* have a head with three hooks, whereas those from *N. fuscus* are variable but have only two very short hooks. Spermatozoa from *Pseudomys forresti*, *P. fumeus*, and *P. gracilicaudatus* all have a head with three hooks. Only one very short hook occurs in *P. novaehollandiae*, and there are no hooks in spermatozoa from *P. delicatulus*. *P. shortridgei* has a spatulate sperm head with a large acrosome. Insertion of the sperm tail is lateral in *P. novaehollandiae*, offset basal in *P. delicatulus* and mid-basal in *P. shortridgei*. Principal and end pieces are shorter in the last two species.

*Notomys alexis*, *N. fuscus*, and *N. mitchellii* have very small testes, whereas they are relatively larger and scrotal in *N. cervinus*. In the first three species seminal vesicles and coagulating glands are vestigial or non-existent, whereas in *N. cervinus* they are well developed and weigh up to 150 mg. The possible phylogenetic and functional significance of these differences is discussed.

## Introduction

Phylogenetic relationships between different species of rodents have been investigated by a variety of techniques, all of which have at least some limitations. In several groups of North American and British myomorph rodents spermatozoal morphology and comparative anatomy of the male accessory sex glands have been used (Friend 1936, Bishop & Walton 1960, Arata 1964, Linzey & Layne 1969, 1974). The latter authors concluded that in *Peromyscus* variation in accessory sex glands corresponded well with major taxonomic groupings based on other criteria, whereas variation in spermatozoal morphology did not coincide with the subgeneric groups. Breed & Sarafis (1979) extended a brief report by Illison (1971)<sup>1</sup>; investigated spermatozoal morphology and male reproductive tract anatomy in some species of Australian rodents, and discussed phylogenetic relationships between various genera. They found that within each genus similar spermatozoal morphology occurred apart from *Notomys*. Illison (1971)<sup>1</sup> reported that *Pseudomys shortridgei* has a primitive sperm type, and *P. delicatulus* (as *Leggadina delicatula*) a spermatozoal head with no hooks, so it appears that *Pseudomys*

exhibits considerable spermatozoal diversity; all five species that we investigated had a sperm head with three hooks. Male accessory sex glands were generally similar between the genera investigated, apart from the two species of *Notomys* which differed markedly (Breed & Sarafis 1979). Here spermatozoal morphology of six other *Pseudomys* and two other *Notomys* species is presented, together with an examination of the male reproductive tract from all four *Notomys* species.

## Materials and methods

Spermatozoa were obtained from single adult male individuals of the following species housed at the Institute of Medical and Veterinary Science (I.M.V.S.), Adelaide.

*Notomys cervinus*: Born at University of N.S.W., received 10.IV.79; *N. fuscus*: Laboratory bred at I.M.V.S., *Pseudomys delicatulus*: collected at Shortcut Rd, Nourlangie, N.T. (I.M.V.S. publ. 1017); *P. forresti*: received from University of N.S.W., 10.IV.79 (I.M.V.S. publ. 953); *P. fumeus*: from Arthur Rylah Institute, Melbourne (I.M.V.S. publ. 952); *P. gracilicaudatus*: from School of Biological Sciences, Macquarie University (I.M.V.S. publ. 950); *P. shortridgei*: from Fisheries and Wildlife Division, East Melbourne (I.M.V.S. publ. 951).

In all individuals a small incision was made into the region of the tail of the epididymis under halothane anaesthesia, and a small biopsy taken. Spermatozoa were then squeezed out onto microscope slides previously flooded

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<sup>1</sup> Illison, L. Abstract of paper presented at Aust. Mammal Soc. Meeting vol. 2, No. 8, Dec. 1971.

with glutaraldehyde/formaldehyde/picric acid fixative (see Ito & Karnovsky 1968). Semi-permanent mounts were made by placing a coverslip on top of the sperm and ringing the mount with DePeX. Subsequently the sperm were photographed with Nomarski optics at CSIRO Division of Horticultural Research, Glen Osmond. Measurements were made of several spermatozoa from each species under phase contrast using an eyepiece micrometer as described by Breed & Sarafis (1979). Although difficulty was experienced in obtaining accurate measurements, the mean values for head length, midpiece, and principal together with endpiece were determined.

Adult male individuals of *Notomys mitchelli*, *N. alexis*, *N. cervinus* and *N. fuscus* collected in the field (1960-1978), preserved in formalin or 70% alcohol, and lodged at the S.A. Museum, W.A. Museum or I.M.V.S., were weighed. A single testis, seminal vesicles with coagulating glands, and ventral prostates

were dissected out, cleared of adherant fat, subsequently weighed and, where appropriate, measured. The testis weight was subsequently doubled to give the approximate weight of paired testes. A few laboratory bred adult *Notomys* held at I.M.V.S. or Medical School, University of Adelaide, were also killed and male reproductive tracts dissected out and weighed. A single *N. cervinus* (provided by Mr R. Briggs) was weighed, dissected and investigated similarly.

### Results

Table 1 and Fig. 1 present morphological details of spermatozoa determined by light microscopy. Sperm of *P. forresti*, *P. fumeus*, and *P. gracilicaudatus* had a head with three hooks, although the size of the sperm head and length of hooks was greater in *P. gracilicaudatus*. The sperm head of *P. novaehollandiae* had a single short top hook and a truncated or non-existent lower hook. That of

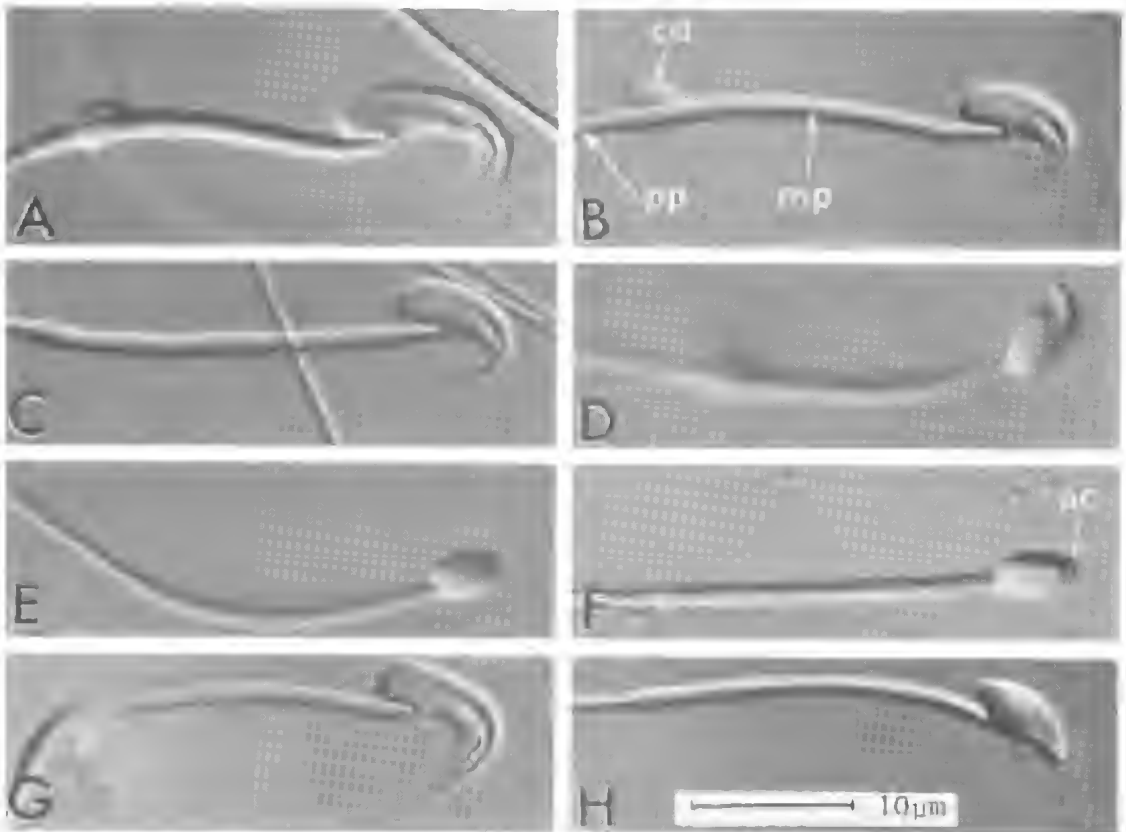


Fig. 1. Spermatozoa. A: *Pseudomys gracilicaudatus*; B: *fumeus*; C: *P. forresti*; D: *P. novaehollandiae*; E: *P. delicatulus*; F: *P. shorridgeli*; G: *Notomys cervinus*; H: *N. fuscus*. mp = middle piece, pp = principal piece, ac = acrosome, cd = cytoplasmic droplet.

TABLE 1: head and tail length of spermatozoa from various *Notomys* and *Pseudomys* species.

Species	Average size of spermatozoa ( $\mu\text{m}$ )			
	Head	Midpiece	Principal and endpiece	Total
<i>Notomys fuscus</i>	7	22	77	106
<i>N. cervinus</i>	9	26	80	115
<i>Pseudomys delicatulus</i>	4	19	65	88
<i>P. forresti</i>	7	26	95	128
<i>P. fumeus</i>	8	20	100	128
<i>P. gracilicaudatus</i>	10	21	98	129
<i>P. novaehollandiae</i>	6	22	78	106
<i>P. shorridgei</i>	5	22	69	96

*P. delicatulus* had no hooks and was smaller than the others. *P. shorridgei* also had sperm with no visible hooks. It was spatulate in shape, and a large acrosome occurred over the nucleus. The principal and endpieces were shorter in the last two species.

*Notomys fuscus* had variable sperm head morphology, but usually there were very short and truncated top and lower hooks. *N. cervinus* sperm had a head with a long top hook and two lower hooks united at their base.

In wild caught *N. alexis* testes weight ranged from a mean of 20-37 mg with time of year. Often no obvious scrotum was discernible. Seminal vesicles were at most only just visible in the preserved material and did not exceed 3 mm length. Coagulating glands and dorsal prostates were vestigial or non-existent. Large ventral prostates occurred which varied in weight during the year; no seasonal trend was apparent. Laboratory bred adult *N. alexis* have similar reproductive tract anatomy (Breed 1979, Breed & Sarafis 1979).

Similar male reproductive tract morphology occurred in *N. mitchellii* and *N. fuscus* (Table 2). By contrast, that of *N. cervinus* was markedly different (Table 2, Fig. 2). The testes were relatively larger and scrotal in position, although adult body weights were similar to the other species. Conspicuous seminal vesicles and coagulating glands were present; their average weight being about 130 mg and length about 10 mm.

#### Discussion

The suggestion by Breed & Sarafis (1979) of intrageneric differences in spermatozoal morphology and male reproductive anatomy in *Pseudomys* and *Notomys* has been confirmed. Most *Pseudomys* and two of four species of *Notomys* have spermatozoa with three hooks.

In *P. novaehollandiae* there appears to be only one short truncated hook, and all three hooks are missing in spermatozoa from *P. delicatulus* and *P. shorridgei*. Preliminary transmission electron microscopy (TEM) has confirmed a large, somewhat eccentrically placed, acrosome

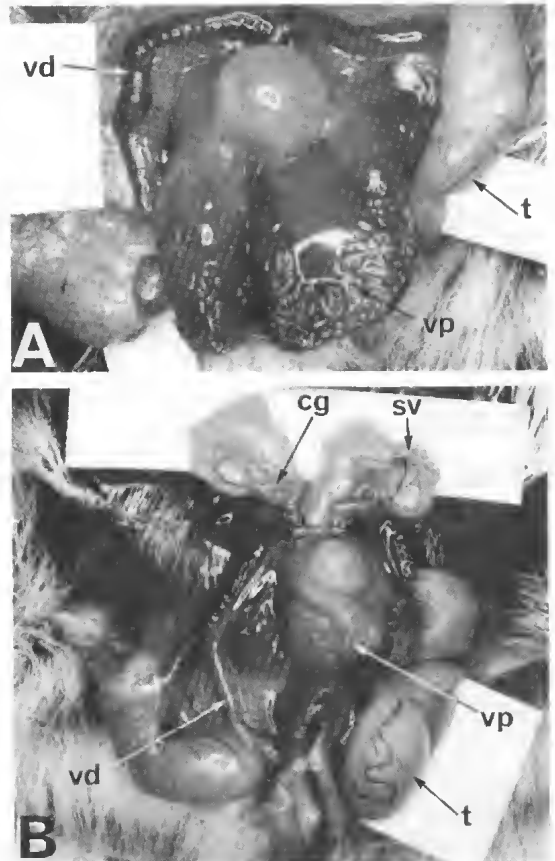


Fig. 2. Male reproductive tracts. A: *Notomys mitchellii*; B: *N. cervinus*. t = testis, sv = seminal vesicle, vp = ventral prostate, cg = coagulating gland, vd = vas deferens.

TABLE 2: Adult reproductive organ weights of male *Notomys* (mean  $\pm$  S.E.)

	No. of animals	Body wt (g)	Testis wt (mg)	Ventral prostrate wt (mg)	Seminal vesicle & coagulating gland wt (mg)
<i>Notomys alexis</i>					
Fixed tissue					
Month of capture					
Jan & Feb	6	20****	22 $\pm$ 4	62 $\pm$ 26	—*
Mar & Apr	16	30 $\pm$ 0.5	31 $\pm$ 6	84 $\pm$ 6	
May & June	19	30 $\pm$ 3	37 $\pm$ 4	77 $\pm$ 9	
July & Aug	18	30 $\pm$ 3	22 $\pm$ 3	55 $\pm$ 2	
Sept & Oct	4	31 $\pm$ 3	30 $\pm$ 1	125 $\pm$ 30	
Nov & Dec	3	37****	31 $\pm$ 11	98 $\pm$ 26	
Fresh tissue	†				
	3	28 $\pm$ 2.6	38 $\pm$ 3	89 $\pm$ 8	—*
	***	29 $\pm$ 2	33 $\pm$ 9	97 $\pm$ 20	
<i>Notomys mitchellii</i>					
Fixed tissue	13	39 $\pm$ 2	50 $\pm$ 3	162 $\pm$ 21	—*
Fresh tissue	3	48 $\pm$ 2	78 $\pm$ 7	407 $\pm$ 223	
<i>Notomys fuscus</i>					
Fixed tissue	9	28 $\pm$ 2	43 $\pm$ 4	58 $\pm$ 17	—*
<i>Notomys cervinus</i>					
Fixed tissue	12	38 $\pm$ 2	133 $\pm$ 8	78 $\pm$ 7	130 $\pm$ 21
Fresh tissue	1	32	170	103	(10 $\pm$ 0.7)*****
					150
					(11)*****

\* Vestigial or non-existent; maximum length of seminal vesiclaes — 3 mm.

† From Breed 1979 (80 days of age).

\*\*\* From Breed & Sarafis 1979.

\*\*\*\*\* Body wt of only one animal recorded.

\*\*\*\*\* Length of seminal vesicles (mm) given in parenthesis.

in *P. shortridgei*, and a few cup-shaped evaginations in the apical part of the nucleus. In *P. delicatulus* the acrosome appears considerably different and somewhat complex in structure.

TEM has also shown that the connecting piece of the tail in *P. shortridgei* has a mid-basal insertion into the sperm head, an offset basal insertion in *P. delicatulus* and a lateral insertion in *P. novaeollandiae* and other species with the more typical sperm head morphology. *N. fuscus*, *N. alexis*, and *N. mitchellii* all appear to have somewhat variable sperm head morphology and the former two species truncated, or nonexistent, hooks (see also Breed & Sarafis 1979).

Most non-Australian murid rodents investigated have a sperm head with a single top hook (vide Friend 1936, Bishop & Walton 1960), whereas most species of Australian rodents have sperm heads with at least two, and usually three, hooks (Illison 1971, Breed & Sarafis 1979). It seems plausible that some

ancestral, non-Rattus, murid rodent evolved a sperm head with this complex structure, and that adaptive radiation of the group within Australia then occurred, most species maintaining this specialised sperm head morphology. The reduction, or lack of, hooks in the few *Notomys* and *Pseudomys* species is, therefore, probably a recently derived state. Variability in sperm head morphology in the two *Notomys* species with truncated hooks may support this contention.

The anatomy of the male accessory sex organs of *Pseudomys* investigated is similar, regardless of the differences in spermatozoal morphology. There are relatively large 'leaf-like' seminal vesicles 6.5 mm or more in length in *P. gracilicaudatus* and *P. australis* (Taylor & Horner 1972), *P. hermannsburgensis* (Taylor & Horner 1970), *P. apodomoïdes*, *P. forresti*, *P. novaeollandiae*, and *P. delicatulus* (Breed, unpublished). Taylor & Horner (1972) state that no coagulating glands follow the posterolateral contour of the seminal vesicles in *P.*

*australis*, but that in laboratory-bred animals such glands were found, and the mixing of secretions of seminal vesicles and coagulating glands results in the occurrence of hard gelatinous material. Vaginal plugs, which in common laboratory rodents result from a mixture of secretions from seminal vesicles and coagulating glands (Mann 1964), have been recorded in laboratory bred *P. australis* (Smith, Watts & Crichton 1972).

In *Notomys* considerable inter-specific differences occur in accessory sex gland and testicular development. *N. cervinus* has the typical complement of male accessory sex glands, whereas in the other species the seminal vesicles, coagulating glands, and dorsal prostates are vestigial or non-existent. *N. cervinus* is also the only species of *Notomys* for which a vaginal plug has been recorded (Crichton 1974) and it also has typical scrotal testes, whereas they are relatively much smaller and perianal in position in the others. Lack of testicular development presumably results in the absence of an obvious scrotum that often occurs in these species.

The above findings indicate, therefore, that spermatozoa and the male accessory sex glands are somewhat labile structures, as considerable variation occurs within *Notomys* that, on other morphological and physiological grounds, appears to be a discrete and closely related group. Since *N. cervinus* has the more conventional pattern of male reproductive tract anatomy, the occurrence of the vestigial glands, very small

testes, and the apparent lack of vaginal plug formation in the other three species is likely to be a recently derived state. The functional significance of these differences has yet to be elucidated, but it may be significant that relative testis size appears to correlate with seminal vesicle and coagulating gland development. Perhaps in species with relatively small testes, fewer sperm are produced, stored, and thus released at ejaculation. Less energy, and therefore seminal fluid, would thus be required for their metabolism in the female reproductive tract. This, in turn, may result in atrophy of some of the glands involved in the production of seminal fluid. Why such divergence should evolve in a closely related group of species is, at present unknown, but a study on the sexual and social behaviour of these animals is being carried out, and this may shed some light on the significance of these observed anatomical differences.

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# FOSSILIFEROUS LOWER DEVONIAN BOULDERS IN CRETACEOUS SEDIMENTS OF THE GREAT AUSTRALIAN BASIN

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## Summary

During 1977-78, 32 fossiliferous Lower Devonian quartzite boulders were discovered within Mesozoic sediments along the southwestern margin of the Great Australian Basin. Previously only two such specimens had been discovered in South Australia though similar occurrences have been known in New South Wales since 1898. Fossils not previously recorded in S.A. include the fish *Wuttagoonaspis*, the brachiopods *Howellella jaqueti* and *Sphaerirhynchia* sp.; the bivalves *Leptodesma inflatum*, *Sanguinolites* sp. and *Praectenodonta* sp.; the gastropod *Strapollus culleni*; and abundant tentaculitids. Similar fossiliferous Devonian rocks are not known in situ in S.A. The probable source area is the fossiliferous Amphitheatre and Mulga Downs Groups near Cobar in N.S.W. It is suggested that boulders were transported to S.A. during the Permian glaciation and then reworked into Cretaceous bouldery shales and sands. All but two of the boulders are found within conglomeratic sediments at the base of the Bulldog Shale. Theories on transport processes during the Cretaceous are discussed; it is concluded that conglomeratic sediments at the base of the Bulldog Shale are reworked submarine debris-flow deposits.